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# Induction of transverse polarity by blue light: An all-or-none response

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**Abstract.** Phototropic stimulation induces a spatial memory. This was inferred from experiments with maize (*Zea mays* L.) coleoptiles involving opposing blue-light pulses, separated by variable time intervals, and rotation on a horizontal clinostat (Nick and Schäfer, 1988b, Planta **175,** 380–388). In those experiments, individual seedlings either curved towards the first or towards the second pulse, or they remained straight. Bending, if it occurred, seemed to be an all-or-none response. Intermediates, i.e. plants, bending only weakly, were not observed. In the first part of the present study it was attempted to create such intermediates. For this purpose the strength of the first, inducing, and the second, opposing, pulse was varied. The result was complex: (i) *Individual* seedlings maintained the all-or-none expression of spatial memory. (ii) However, on the level of the whole *population,* the time intervals at which a given response type dominated depended on the fluence ratio. (iii) Furthermore, the final curvature was determined by the fluence ratio. These results are discussed in terms of a blue-light-induced transverse polarity. This polarity initiates from a labile precursor, which can be reoriented by an opposing stimulation (indicated by the strong bending towards the second pulse). The strong curvatures towards the first pulse over long time intervals reveal that, eventually, the blue-light-induced transverse polarity becomes stabilised and thus immune to the counterpulse. In the second part of the study, the relation between phototropic transduction and transverse polarity was characterised by a phenomenological approach involving the following points: (i) Sensory adaptation for induction of transverse polarity disappears with a time course similar to that for phototropic sensory adaptataion. (ii) The fluence-response for induction of transverse polarity is a saturation curve and not bell-shaped like the curve for phototropism. (iii) For strong counterpulses and long time intervals the clinostat-elicited nastic response (Nick and Schäfer 1989, Planta **179,** 123–131) becomes manifest and causes an "aiming error" towards the caryopsis. (iv) Temperature-sensitivity of polarity induction was high in the first 20 min after induction, then dropped sharply and rose again with the approach of polarity fixation. (v) Stimulus-summation experiments indicated that, for different inducing fluences, the actual fixation of polarity happened at about 2 h after induction. These experiments point towards an early separation of the transduction chains mediating phototropism and transverse polarity, possibly before phototropic asymmetry is formed.

**Key words:** Blue light (polarity induction) – Coleoptile – Phototropism – Polarity (transverse) – Signal transduction – *Zea* (phototropism)

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## Introduction

Phototropic stimulation, in maize coleoptiles, evokes two distinct responses: (i) tropistic curving towards the stimulus, visible after a delay of 20–30 min (Iino 1988); and (ii) induction of a spatial memory emerging from a labile precursor after a lag of 80–90 min (Nick and Schäfer 1988b). This was concluded from experiments involving a first, inducing blue-light pulse, and a second, opposing pulse of equal fluence at variable time intervals later. In order to exclude distortions caused by asymmetric gravity influences, the plants were kept on a horizontal clinostat. For short time intervals, under these conditions, a counterpulse was able to overwhelm the effects of the first, inducing pulse, yielding final curvatures of about 150° towards the second pulse. This change was assumed to mirror the lability of spatial memory in its early stage. If the time interval exceeded 90 min, however, the opposing pulse produced only a transient bending response, which ceased after a while and was replaced by a renewed, strong orientation according to the first stimulation. The final curvatures (more than 90°) did not reveal any traces that a counterstimulation had taken place. This behaviour was accepted as evidence for the fixation of spatial memory. For time intervals during which the transition from lability towards stability was to be expected, together with those two bending responses, a third

response type occurred, where coleoptiles did not bend in either direction. This was seen as "tie-game" situation, where the contradictory effects initiated by the opposing stimuli supposedly had cancelled each other. Apparently, the spatial memory was expressed in a discontinuous pattern, with individual seedlings choosing between either strong bending towards the second or towards the first stimulation, or not bending at all. The response of *individual* seedlings did not reveal intermediates of any kind, i.e. plants, curving only weakly, were not found. Nevertheless, it was possible to construct a smooth, sigmoidal curve, if the average final curvature was plotted against the time interval elapsed between the pulses. From this curve the time, at which the spatial memory became stable, was estimated to be 85 min. However, the standard deviations in this transitional range were exorbitant. By constructing frequency distributions of curvature for the different regions of the curve, the probability, by which a given seedling chose one of the three responses described above, was shown to depend on the time interval between the light pulses. Once the decision was taken, however, it was expressed as all-or-none response. The large standard deviations for a time interval of 85 min resulted from the simultaneous presence of all three response types. For shorter time intervals strong bending towards the counterpulse, for longer time intervals strong bending towards the inducing pulse, dominated. A similar logical situation was encountered when analysing the interaction between phototropism and the clinostat-elicited, dorsoventral, nastic bending (Nick and Schäfer 1989), the inversion of gravitropism by symmetric irradiation with blue light on the clinostat (Sailer et al. 1990), and the interaction between photo- and gravitropically induced spatial memories (Nick et al. 1990).

From these studies, long-term effects of tropistic stimulation are concluded to be often all-or-none phenomena. In such responses, the individual decides between a few discrete outputs, although the stimulus occurs in a graded manner. Hence, the size of a given output does not depend on the strength of induction. However, the probability (actually, the frequency) by which that output is chosen is gradually determined by the amount of the eliciting agent. It has been proposed that blue-light-induced spatial memory mirrors a stable *transverse polarity* (Nick and Schäfer 1988b), and this all-or-none behaviour was cited as supporting evidence. However, hitherto, both stimulations were kept constant – fluences inducing maximal first positive phototropism were used throughout. It might therefore well be that the invariant response of individual seedlings is the consequence of a constant input level rather than a real all-or-none phenomenon. By means of frequency distributions of final curvature we have tested whether the all-or-none behaviour during curvature expression still holds if the input level is varied. If this process is, indeed, a case of polarity induction in sensu strictu, one should obtain qualitatively identical results if the fluences of inducing and testing pulses are varied. The problem can be condensed into the questions: Is spatial memory per se an all-or-none process or is it possible to create graded outputs of individual seedlings? Is it possible to induce "partial" polarities? To answer these questions is the aim of the present paper.

## Material and methods

*Plant material and light sources.* Maize (*Zea mays* L. cv. BRIO42HT; Asgrow, Bruchsal, FRG, stored in the dark at 3° C) seedlings were grown for experimental use as described elsewhere (Nick and Schäfer 1988a, b). In brief, they were kept for 2 d under 0.2 $W \cdot m^{-2}$ red light, so as to suppress mesocotyl growth and nutations (Kunzelmann and Schäfer 1985), and then transferred to darkness until the beginning of the experiments. From stimulation onset until harvest, 1 d later, the whole experiment was performed in a red background illumination in order to avoid distortions caused by blue-light-induced phytochrome gradients (Hofmann and Schäfer 1987). Further details of light sources and measurements are given in Nick and Schäfer (1988a, b).

*Stimulation treatments.* The plants were subjected to three types of experimental procedures, designated standard, stimulus-summation, and temperature-sensitivity schedules. In the standard schedule, a blue-light pulse of variable fluence was administered unilaterally within the plane of the longer coleoptile cross-section. Then, seedlings were positioned onto a horizontal clinostat and rotated at 0.5 rpm, irradiated with a second pulse of variable fluence from the opposite direction after given time intervals, and eventually returned to the clinostat where they remained until harvest (1 d after stimulation onset). Special care was taken to minimize uncontrolled stimulation by gravity. Therefore, the glass vials containing the seedlings were mounted on the already-moving clinostat, and irradiation time was restricted to 30 s. The short irradiation times had the additional advantage of excluding the influence of pigment desensitisation (Iino 1987). The gravity stimulus perceived during the application of the counterpulse (seedlings, because of induction by the first pulse, often already displayed phototropic bending), was certainly too small to account for the large effects described in this work. Moreover, the sensory and transductional adaptation experienced during the preceding clinostat treatment should have prevented any effective gravity perception (Pickard 1972), a suggestion that was confirmed by control experiments (data not shown). For the stimulus-summation schedule, the second pulse was replaced by two pulses with an identical direction but half the fluence of the pulse they replaced. Again, the time interval between those "semi-pulses" was subject to variation. The temperature-sensitivity schedule included a cold period (5° C or 15° C) of variable length at variable time intervals after the first pulse. Except for this cold treatment, the experimental set-up was the same as for the standard an stimulus-summation schedules.

*Response evaluation.* Twenty-four hours after stimulation onset, shoots were excised and curvature measured as described in Nick and Schäfer (1988a). Curvatures directed towards the first stimulus were defined as positive. In cases where seedlings showed "aiming errors" with a response direction deviating from the stimulation plane (the longer coleoptile cross-section), the response was additionally specified by the bending azimuth. Curvatures towards the caryopsis were indicated by azimuths of 180°, positive bending within the stimulation plane by 90° (Nick and Schäfer 1989). Time courses of stabilisation were constructed for a given pair of pulses by plotting mean final curvature versus the time interval elapsed between the pulses. Such plots comprise data from about 120–200 plants, with each point representing the average of 6–16 seedlings. Bars indicate standard deviations. In order to test whether the all-or-none behaviour of curvature expression persisted for the negative, the intermediate, and the positive regions of these curves, frequency distributions of final curvature (classes of 20° width) were established from an independent set of 50–90 seedlings. In the case of "aiming errors", the angular component parallel to the stimulation plane was additionally calculated (dashed curves in Fig. 8a).

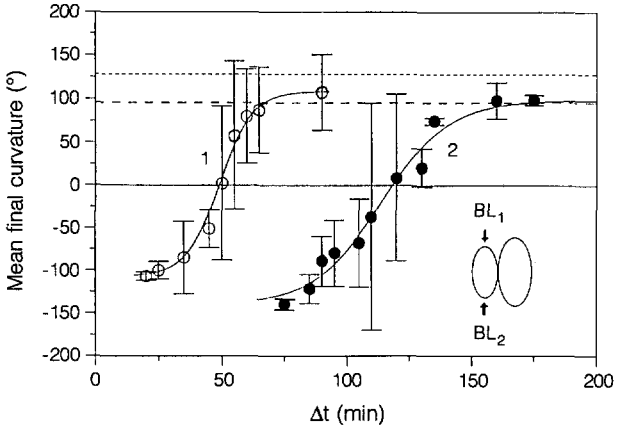


**Fig. 1.** Stabilisation time course for different pairs of stimuli. *Curve 1:* inducing pulse 1.9 μmol · m$^{-2}$, opposing pulse 0.85 μmol · m$^{-2}$. *Curve 2:* inducing pulse 0.85 μmol · m$^{-2}$, opposing pulse 1.9 μmol · m$^{-2}$. Maize plants were kept under saturating, symmetric red light (2.5 W · m$^{-2}$) on a clinostat. *Dotted line:* final curvature for a time interval of 4 h *(curve 1). Dashed line:* the same for *curve 2. Error bars* indicate SDs. The ellipses depict the arrangement of stimuli with the smaller ellipse symbolising the coleoptile cross-section, and the larger ellipse denoting the position of the caryopsis. *Positive values* mean curving towards the inducing pulse

Temperature sensitivity was characterised by the $Q_{10}$, estimated from the time shift between the stabilisation time course with cold treatment (5° C or 15° C) compared to the control (25° C).

## Results

*Typical spatial-temporal pattern.* Even for very different pairs of light pulses, the time course of stabilisation displays similar characteristics despite a shift in time (Fig. 1). Mean final curvature, plotted versus the time interval elapsed between the pulses, follows a sigmoidal curve. For short time intervals, values are negative, for long time intervals positive. The standard deviations in the midpoints of the curves are extraordinarily large. The reason for this is the three distinct subpopulations (Fig. 2a–c), peaking at negative, zero, and positive curvatures, and separated by broad troughs. For each pair of stimulations, the position of these peaks is constant (Fig. 2a–c, dotted and dashed lines). However, the size of the peaks depends on the time interval between inducing and opposing pulses: for short time intervals the dominating peak is negative (Fig. 2a), for long time intervals it is positive (Fig. 2c). For intermediate time intervals, all three peaks are present (Fig. 2b). By averaging over these three subpopulations, very large standard deviations are obtained for the situation shown in Fig. 2b, i.e. for intermediate time intervals. Here, all three peaks are conspicuously manifest. A semilogarithmic plot of peak position versus fluence ratio (Fig. 3) follows a pair of parallel straight lines, which ascend, if the first pulse gains strength over the counterpulse, i.e. the stronger the first pulse, the stronger is the bending response of individual seedlings towards the first pulse for long time intervals, and the weaker is the movement towards the counterpulse, for short time intervals. The difference

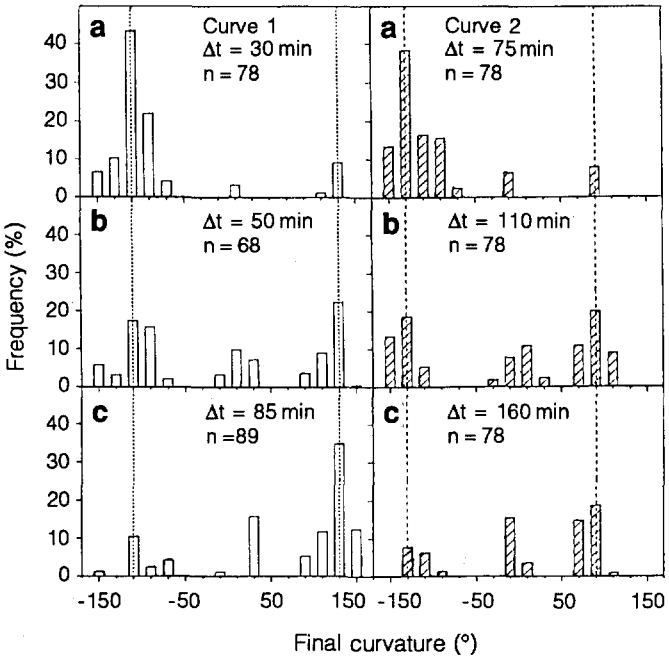


**Fig. 2a–c.** Frequency distribution of final curvature for the curves of Fig. 1. *Open bars* correspond to curve 1, *hatched bars* to curve 2. **a** Negative region of the stabilisation time course (time intervals 30 min and 75 min, respectively). **b** Transition range (time intervals 50 and 110 min, respectively). **c** Positive region of the curve (time intervals 85 and 160 min, respectively). *Positive values* indicate curvature towards the inducing pulse. The *dotted* (curve 1) and *dashed* (curve 2) lines stress the position of the frequency peaks. For a given pair of stimuli they are constant; however, they differ between different pairs of stimuli

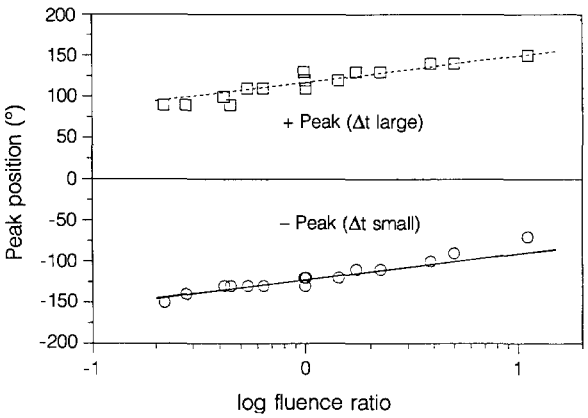


**Fig. 3.** Plot of the positions of the frequency peaks (defined as the vertical lines in Fig. 2) versus the logarithm of the fluence ratio (inducing and opposing pulse). *Positive angles* stand for curvatures towards the inducing stimulus. Each point represents data from 70 to 120 plants

between positive and negative peaks amounts to 240°, and is apparently independent of the fluence ratio. For the interpretation of Fig. 3 see the first part of the *Discussion.*

Whereas qualitatively the all-or-none character of the spatial pattern remains unaffected by changes of stimulation strength (Fig. 2a–c), there is a quantitative depen-

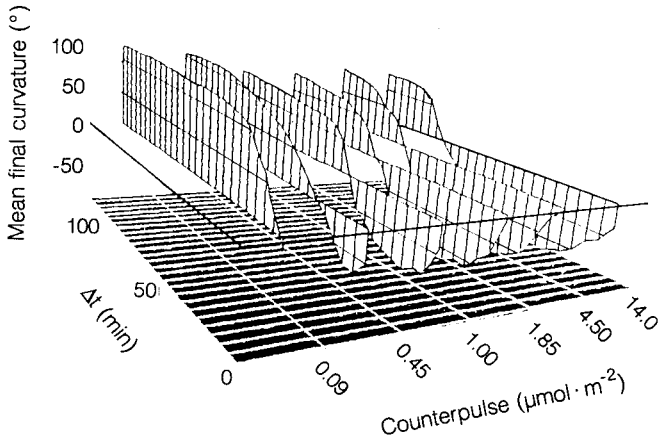


**Fig. 4.** Stabilisation time course for a given inducing pulse of 1.9 μmol · $m^{-2}$ and variable counterpulses. For the sake of clearness only the fitted curves are shown. Each curve represents data extracted from 120–200 plants

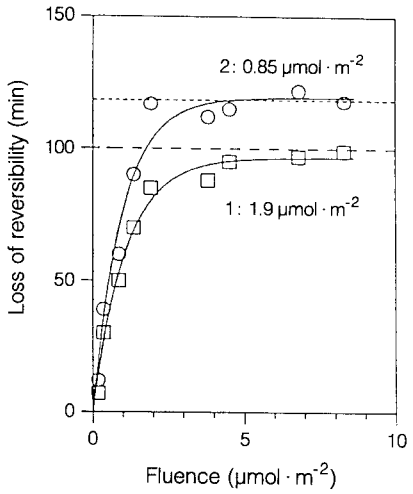


**Fig. 5.** Fluence-response curve for the ability of counterstimulation to reverse the spatial memory induced by a first pulse of 1.9 μmol · $m^{-2}$ *(curve 1)* or 0.85 μmol · $m^{-2}$ *(curve 2)*. Each point mirrors one entire time-course of stabilisation, i.e. the data from 120–200 individual seedlings. The *dotted* and *dashed lines* give the saturation, as calculated from the curve fitting

dence on fluence ratios of the final curvature (Fig. 3) and of the temporal pattern of the response (Fig. 1): the time-courses of stabilisation are shifted in time for different pairs of stimulations, although maintaining their sigmoidal shape. If the inducing pulse is kept constant, with the fluence of the opposing pulse raised, the time at which the negative runs into the positive branch of the curve (taken as a measure for the stabilisation time), is more and more delayed (Fig. 4). Additionally, in agreement with Fig. 3, the negative branch becomes more negative, the positive branch less positive. In order to analyse this behaviour in more detail, the stabilisation times (transitions from the negative to the positive branches of the curves) for two inducing pulses were plotted against the fluence of the counterpulse (Fig. 5). This plot shows for how long a counterpulse, whose fluence is given on the abscissa, is able to reverse the spatial memory induced by the first pulse, i.e. it is the fluence-response curve for the reversion caused by the opposing light pulse. This relation can be described by a curve with a steep initial ascent and saturation for higher fluences. If the first pulse is optimal with regard to first positive phototropism, the curve levels off at about 100 min. For a suboptimal inducing pulse, however, saturation takes effect at about 120 min. Despite these differences in the saturation level, the counterpulse fluence at which this level is reached (about 2 μmol · $m^{-2}$) appears to be fairly independent of the inducing fluence. A further increase of the counterpulse fluence does not improve the capability of the opposing pulse to reverse the spatial memory.

The same data, however, read in a different way, relate the fluence of the counterpulse to the time up to which it is able to reverse the spatial memory. Whereas the spatial memory induced by a given first pulse escapes disruption by the opposing pulse early if the counterpulse is weak, for a stronger counterpulse it needs more time to do so. Thus, in addition, this kind of graph defines how stable the spatial memory has become at a given time point. Hence, stability is measured in terms of the counterpulse fluence which is necessary for the reversion of the spatial memory. For phototropically optimal stimulation this stability evolves faster than for suboptimal stimulation. However, in both cases there exists a threshold time at which the stability of the spatial memory grows steeply and, apparently, to irreversible levels. This time is reached earlier (at about 100 min) for optimal induction, whereas suboptimal induction needs about 20 min longer. The curves of Fig. 5 indicate that spatial memory is irreversibly fixed at 100–120 min. Alternatively, they could be explained by a saturation of the counterstimulus effect at 2 μmol · $m^{-2}$. In order to discriminate between the two possibilities, stimulus-summation experiments were undertaken.

*Stimulus-summation experiments.* A putative saturation of counterpulse effects, if situated in the early phase of the signal chain, might be circumvented by application of two strong counterpulses: as soon as the saturation produced by the firs counterpulse has faded away, it should be possible to trigger the same signal chain again by a second counterpulse. If such a treatment were successful, the time limit for the reversion of spatial memory should be shifted beyond 100 min or 120 min. The time-course of stabilisation, thus obtained, turned out to be complex (Fig. 6a, b). The pattern for phototropically optimal stimulation was somewhat different from that for suboptimal stimulation. Therefore, by way of an example, the resulting curves are described for induction by 1.9 μmol · $m^{-2}$ (Fig. 6a) and 0.85 μmol · $m^{-2}$ (Fig. 6b). If an inducing pulse of 1.9 μmol · $m^{-2}$ is followed 90 min later by a counterpulse of equal strength, final curvature follows the first stimulation (Fig. 6a, dashed line). If the counterpulse is immediately afterwards supplemented by a pulse, equal in strength and direction, no effect of the second counterpulse upon final curvature can be detected. If, however, the second counterpulse is given only 105 min after induction (i.e. 15 min after the first counterpulse), the final curvature is smaller, and eventually

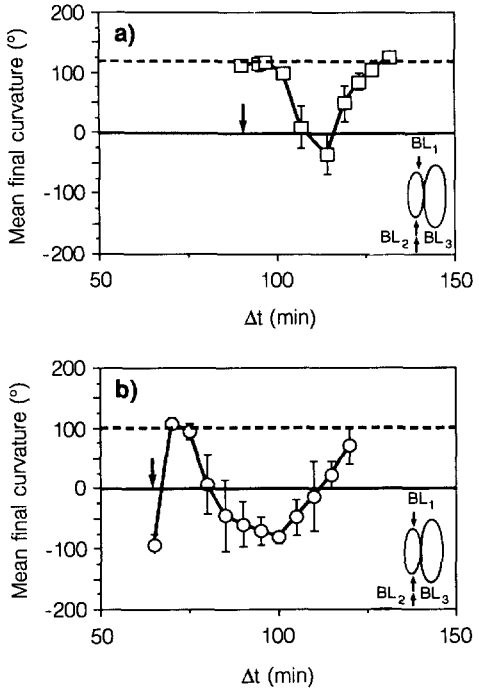


**Fig. 6a, b.** Time-course of stabilisation for phototropically optimal (**a**) and suboptimal (**b**) stimulation under the conditions of the stimulus-summation schedule. **a** Inducing pulse 1.9 μmol · $m^{-2}$, first counterpulse 1.9 μmol · $m^{-2}$ (90 min after induction), second counterpulse 1.9 μmol · $m^{-2}$ at variable time intervals after the first counterpulse. **b** Inducing pulse 0.85 μmol · $m^{-2}$, first counterpulse 0.85 μmol · $m^{-2}$ (65 min after induction), second counterpulse 0.85 μmol · $m^{-2}$ at variable time intervals after the first counterpulse. *Dashed lines* indicate final curvature obtained, if only the first counterpulse was applied. *Positive values* indicate curving towards the inducing pulse. Each point represents the average from eight seedlings, *error bars* have to be read as standard deviations. The abscissa indicates the time interval elapsed after the induction by the first pulse. *Arrows* give the time of the first counterstimulation

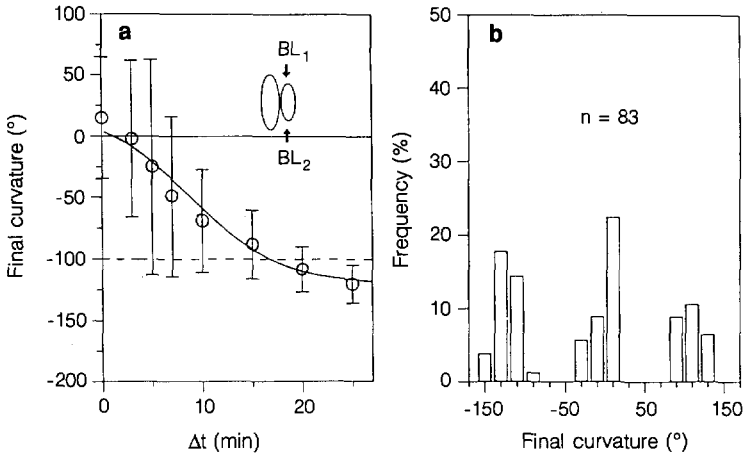


**Fig. 7. a** Initial phase of the stabilisation time course of Fig. 1, curve 1. *Error bars* indicate standard deviations. Each point represents the mean from 12 individual plants. *Dashed line:* mean final curvature for a time interval of 40 min (compare Fig. 1, curve 1). **b** Frequency distribution of final curvature integrated over time intervals between 0 and 20 min for the curve shown in **a**

even slightly negative mean values occur (Fig. 6a). Extending the time interval until the second counterpulse to more than 115 min after induction makes the curve rise anew and yield positive values (final curvatures towards the first, inducing, pulse).

Except for the first 5 min, the pattern for an inducing pulse of 0.85 μmol · $m^{-2}$ looks similar, but is shifted in time (Fig. 6b). If the second counterpulse follows 5–15 min after the first counterpulse, final curvatures are positive. They become negative for longer time intervals, but eventually rise again to positive values if the second counterpulse is given very late after the first. If the second counterpulse follows the first immediately, the curvature is dominated by the counterstimulation, which is in contrast to the situation of Fig. 6a.

Thus, the counterpulses enhance each other, even for a situation where, according to Fig. 5, the first counterpulse is supposed to be saturating. The time at which spatial memory is fixed seems to be later than 100–120 min: at a time when the spatial memory induced by the first given pulse appears to be stable against a given counterstimulation it is, nevertheless, not irreversibly fixed. The data plotted in Fig. 5, therefore, mirror only apparent fixation points. The final transition towards positive curvatures in the stimulus-summation experiments is certainly the more accurate estimation for the actual fixation of spatial memory. This time, referred to as the actual fixation point, was determined for different combinations of inducing and opposing pulses (Table 1). It was found to be around 2 h, irrespective of the inducing fluence. Whereas a phototropically optimal first pulse establishes a spatial memory considerably faster than a half-optimal pulse (Fig. 5b), the time at which this memory becomes irreversibly fixed (Table 1) appears to be more independent of the inducing fluence.

*Deviations from the standard spatial-temporal pattern.* For very short time intervals, and for phototropically superoptimal counterpulses, conspicuous deviations from the standard spatiotemporal pattern (Figs. 1–4) resulted. When the counterpulse was applied immediately after the inducing pulse, using the standard schedule (Fig. 7a), mean final curvature was close to zero. It then became negative for increasing time intervals, and reached the values shown in Fig. 1 (Fig. 7a, dashed line) if the time interval exceeded 25 min. Again, the standard deviations in this region were very large, but they decreased as the final curvature approached its negative climax. A frequency-distribution plot for time intervals between 0 and 20 min again revealed three peaks separated by broad troughs (Fig. 7b). The positions of the peaks corresponded to those found for longer time intervals (Fig. 2a–c, open bars).

When the counterpulse was superoptimal with respect to first positive phototropism, a second deviation from the standard pattern was detected (Fig. 8a, b). For short time intervals, a strong, negative curvature was observed, whereas plants curved towards the caryopsis for long time intervals. When the curvature component parallel to the stimulation plane was plotted (Fig. 8a, dashed curves) there was almost no difference from total cur-

**Table 1.** Actual and apparent fixation points for different pairs of stimulations. The apparent fixation point is defined as the time interval for which the counterpulse outweighs the inducing pulse. This time can be shifted if the counterpulse is complemented by a second pulse after certain time intervals. The equilibrium under these conditions occurs later, at a time which is supposed to be a better estimate for the actual fixation

| Inducing pulse ($\mu mol \cdot m^{-2}$) | First counterpulse: | | Second counterpulse ($\mu mol \cdot m^{-2}$) | Apparent fixation (min) | Actual fixation (min) |
|---|---|---|---|---|---|
| | $\mu mol \cdot m^{-2}$ | given after | | | |
| 1.9 | 1.9 | 90 min | 1.9 | 85 | 120 |
| 1.9 | 0.85 | 60 min | 0.85 | 50 | 118 |
| 0.85 | 0.85 | 65 min | 0.85 | 60 | 117 |
| 0.85 | 1.9 | 120 min | 1.9 | 117 | 123 |

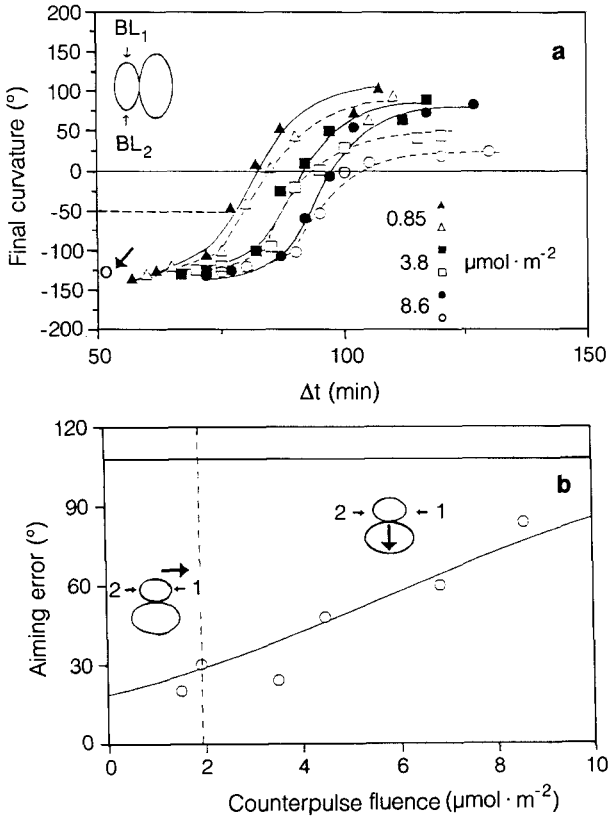


**Fig. 8. a** Stabilisation time-course for induction with 1.9 $\mu mol \cdot m^{-2}$ and phototropically superoptimal counterstimulation. *Solid curves* and *filled symbols* show total curvature, ignoring its orientation. *Dashed curves* and *open symbols* denote the curvature component parallel to the plane of stimulation. Opposing pulses 0.85, 3.8, and 8.6 $\mu mol \cdot m^{-2}$. *Dashed line:* total curvature after application of a counterpulse of 8.6 $\mu mol \cdot m^{-2}$ and omission of the inducing pulse. *Arrow:* final curvature after irradiation with 26.7 $\mu mol \cdot m^{-2}$ from above instead of counterstimulation, 50 min after induction. **b** "Aiming error" of curvature for long time intervals after induction with 1.9 $\mu mol \cdot m^{-2}$. 0° means curvature within the stimulation plane, 90° curvature towards the caryopsis (perpendicular to the stimulation plane). Each point comprises data from 40–60 plants. *Solid line:* "aiming error" for a counterpulse of 26.7 $\mu mol \cdot m^{-2}$. The *large arrows* symbolise the curving direction for the respective curve ranges. *Dashed line:* fluence of the inducing pulse

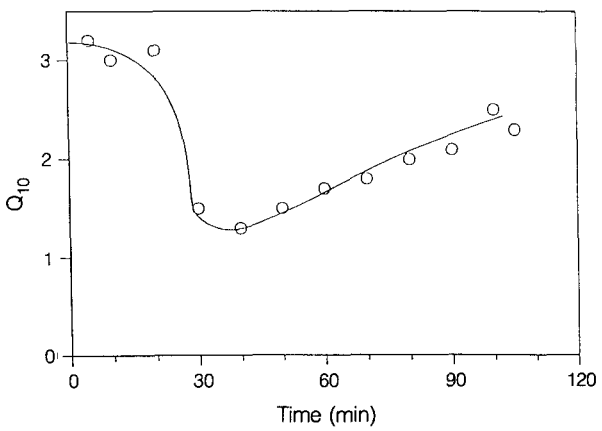


**Fig. 9.** Temperature sensitivity of spatial memory following induction with 1.9 $\mu mol \cdot m^{-2}$ blue light. Each point represents one stabilisation time course comprising data from 50–70 plants. The cold treatment lasted for 20 min starting with the time indicated on the abscissa. In the late part of the curve (beyond 90 min) the cold interval was reduced to 10 min

vature for short time intervals. The plants curved quite precisely towards the counterpulse. For long time intervals, however, the curves became increasingly flatter if the strength of counterstimulation rose. This can be ascribed to the "aiming error" shown in Fig. 8b. The total curvature as such remained unaffected (Fig. 8a, solid curves), it was simply displaced towards the caryopsis. For very high counterpulse fluences, coleoptiles curved towards the caryopsis, i.e. perpendicular to the stimulation plane. It is remarkable that the curvatures in the negative branch of the curve were much larger than those that the counterstimulation could induce if it was not preceded by the inducing pulse (Fig. 8a, dashed line). To put this point more sharply, in one experiment the second stimulation was replaced by light from above, i.e. stimulation without a gradient (Fig. 8a, arrow), which resulted in a strong negative curvature. In other words, blue light from above could reverse the effects elicited by the inducing pulse *(see Discussion)*.

*Temperature dependency.* The $Q_{10}$ of memory formation was followed with time for a phototropically optimal inducing pulse and equal counterstimulation. When the cold treatment intervened later than 90 min after induction, the counterpulse was supported, 20 min later, by a second counterpulse of equal fluence (counteracting the inducing pulse as well). This variation of the stimulus-summation schedule was intended to extend the period during which memory could be reversed. The cold period

**Table 2.** Cold stability of spatial memory induced by 1.9 μmol · $m^{-2}$ of blue light and tested by a counterpulse of equal fluence 90 min after the first pulse. Positive values indicate bending towards the inducing pulse. The control consisted in applying the counterpulse 90 min after the inducing pulse without cold treatment

| Start of cold (5° C) treatment (min) | duration (min) | Mean final curvature (°) |
|---|---|---|
| 60 | 30 | − 76.3 ± 16.8 |
| 120 | 30 | + 82.4 ± 21.2 |
| 120 | 240 | + 97.8 ± 19.2 |
| 180 | 120 | + 105.3 ± 27.2 |
| Control (no cold treatment) | | + 107.3 ± 19.9 |

caused a delay of memory fixation, used to calculate the $Q_{10}$ of memory formation at the time of the treatment. This time course of temperature sensitivity (Fig. 9) uncovered an early sensitive phase (values about 3) lasting until 20 min after induction. Around 40 min, values were low (about 1.5), indicating that relatively temperature-independent processes took place. Then, steadily rising values marked the onset of a further period of increasing sensitivity lasting until the end of the measurable period (2.5 at 110 min), close to the putative time of memory fixation. It should be mentioned that a cold treatment administered *after* the time of fixation could no longer destroy the memory – even if it lasted for up to 4 h (Table 2).

## Discussion

*No "partial" polarities, but graded precursors.* It was not possible to induce intermediates between the three outputs of individual seedlings (Figs. 2a–c). The plants maintained their all-or-none behaviour: strong bending towards the first pulse for long time intervals, strong bending towards the second pulse for short intervals, and zero curvature for intermediate time intervals. The question raised at the end of the *Introduction* has to be answered negatively: there is no "partial" polarity, there are instead, ungraded, clearcut all-or-none decisions. However, is the answer really as unequivocal as just postulated? Certainly not; if Figs. 3 and 4 are taken into account a distinctly graded behaviour is apparent: (i) The time at which the memory attains stability against counterstimulation depends upon the strength of both the inducing and the opposing pulses (Figs. 1, 4, 5). (ii) The curvature a given plant expresses, once it has "chosen" one of the three possible outputs (bending towards the first pulse, bending towards the second pulse, or not bending at all), is not a constant, but determined by the ratio of the fluences involved (Fig. 3). It is felt that these, obviously graded, features of spatial memory are not in harmony with the statement that memory formation is an all-or-none process. A solution of these contradictions is necessary. It requires, however, considering briefly some theoretical aspects of polarity induction.

One fascinating feature of polarity induction, found in most cases investigated hitherto, is the existence of amplification mechanisms. They transform initial, small, graded asymmetries into steep clear gradients in an all-or-none like fashion (Gierer 1981). Models for such transformation processes must meet two preconditions: the extension of the output must be independent of that of the input, and the decision as to which of the possible outputs is selected must depend on the direction of the input. There are various mechanisms whereby such patterns can be attained, but the most fundamental and successful model so far is that postulated by Gierer (1981). The initial asymmetry (stemming from environmental or internal cues or random fluctuations) locally elicits autocatalytical processes. The latter exhibit long-distance lateral cross-inhibition either by diffusion of an actual inhibiting agent or by mutual competition for limited resources maintaining the autocatalytic process. In plants, such models have been successfully entertained for the description of thallus-rhizoid polarity in phaeophycean zygotes and pteridophytean spores (Weisenseel 1979), polarity of regenerating vessels in higher plants (Sachs 1984), and phyllotaxis (Green 1985). One would expect the early stages of such a process to be graded, whereas late steps should be characterised by an all-or-none behaviour independent of the strength of the incoming signal. One might expect the informational content of the system to diminish drastically during this process (Wiener 1963). It is not possible to reverse all-or-none processes without external sources of information. Thus, generally, for all-or-none processes, graded situations should accompany the early states, whereas ungraded, all-or-none responses indicate final states.

The curvature of an individual seedling is defined by two factors. Its direction is determined by all-or-none processes (Fig. 2a–c), possibly involving mechanisms such as those postulated by Gierer (1981). The degree to which the decision for curving becomes manifest, however, is limited. This limitation is related in a graded manner to the ratio of fluences (Fig. 3). It must therefore depend upon the early steps of memory transduction, when the initial information has not yet been erased by extensive amplification processes. This implies a branch of signal transduction into a chain leading to the decision process itself, and a chain regulating to what extent this decision is expressed. This bifurcation will be discussed below in more detail.

At this stage, the second of the contradictions (Fig. 2a–c, Fig. 3) mentioned above, appears to be somewhat alleviated. Still, the graded dependence of stabilisation time upon fluence (Figs. 1, 4, 5) has to be reconciled with the concept of an all-or-none process. This scientific problem is very common and therefore worth a reconsideration. In plants, such transitions from quantitative, graded inputs towards qualitative, ungraded outputs are well-known, for instance from studies of the dependence of germination rate upon phytochrome photoequilibrium (Mohr 1972), of flowering upon daylength (Fukshansky 1981), or the orientation of thallus-rhizoid polarity upon the fluence of the inducing blue light in pteridophytean spores and phaeophycean zygotes (Haupt 1957). In all these cases, although the individual response obeys an all-or-none behaviour (a seed either

germinates or it does not), it is possible to construct "smooth", graded curves, based on the whole population of organisms. Such curves represent probabilistic features of the individual process as means, thresholds or time dependencies. It is obvious that in dealing with such processes one must be aware of whether the logical concepts are extracted from population analysis or the observation of individuals. The curves of Figs. 1 and 4 are, in fact, cumulative frequency distributions over time for the proportion of plants with an already stable memory. Instead of "mean final curvature" it would, in principal, be possible to rename the ordinate "percentage of plants, bending towards the first pulse". Nevertheless, the plot of mean final curvature makes sense because it is necessary for the estimation of stabilisation times plotted in Fig. 5. But one has to be conscious about the fact that the smooth curves mirror probabilities (strictly speaking, frequencies), and are not actually a graded expression of final curvature. Of course, there must be something which is responsible for the gradual increase of the memory-stabilisation frequency. Here again, the early steps of transduction come into play. In other words, the all-or-none step is certainly not placed earlier than about 2 h after induction because until that time it is possible to describe the process by gradually rising curves (Fig. 5, 6a, b).

In conclusion, one can answer the question of "partial" polarities by the statement that, although individual seedlings display an unambigously ungraded polarity, there are graded precursors. The point at which those early gradients are transformed into the eventually observed all-or-none decision is most certainly not earlier than 2 h after induction.

*Relationship to phototropic transduction.* Since the discussion of Figs. 2 and 3 leads to the idea of split signal transduction, the question arises of where the two chains separate (Fig. 10). Of course, one has first to ask whether the two chains share common steps at all. Besides the fact that both appear to be triggered by blue light, supporting evidence can be extracted from Fig. 7a. The positive final curvatures obtained for very short time intervals in a fraction of the population are certainly not due to memory fixation. Otherwise, there should be no negative curvature if time intervals increase. The easiest explanation is that the counterpulse has not been perceived properly owing to sensory adaptation of phototropic perception (Iino 1987). Sensory adaptation is inhomogenous over the population and this inhomogeneity is amplified under the conditions of an all-or-none response, causing the observed large standard deviations. Nevertheless, it is clear that the initial sensitivity is restored within 20 min after stimulation. Then, as the negative curvatures show, the counterpulse is sensed normally, leading to a reversion of the labile early form of memory. Thus, Fig. 7a can be interpreted in terms of a de-adaptation time course for blue-light perception. This time course resembles that for phototropic de-adaptation (Arisz 1915; Briggs 1960; Meyer 1969; Iino 1987). Thus, it seems feasible to assume common perception of phototropic and memory transduction (Fig. 10). However,

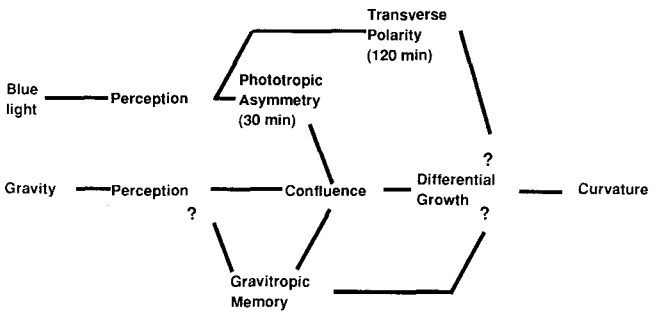


**Fig. 10.** Proposed relationship between transduction chains for phototropism, gravitropism, and polarity

whereas phototropic transduction achieves its end point at about 20–30 min, when curvature becomes perceptible even to the naked eye, memory transduction needs much longer. Its final step, memory fixation, is reached not earlier than about 2 h after induction, as pointed out above. Experiments where curvature and memory transiently contradict each other (Nick and Schäfer 1988b) demonstrate that spatial memory is not simply an extension of the phototropic response but must involve a parallel signal chain. In this context, Fig. 5 is interesting. It mirrors the fluence-response relationship for the counterpulse effect. This curve is clearly not bell-shaped, as is the fluence-response curve of first positive phototropism (Iino 1988; Nick and Schäfer 1988a). It is, instead, a saturation curve. The bell-shaped fluence-response curve of first positive phototropism is usually explained by the perception of the difference across the organ (Kunzelmann et al. 1988). The fluence-response relation for the local light effects are supposed to be saturation curves. These curves are, of course, shifted by the blue-light absorption gradient over the organ cross-section. If such curves are subracted from each other, i.e. if phototropic asymmetry is established (Buder 1920), a bell-shaped curve results. Thus, the phototropic fluence-response curve mirrors the construction of phototropic asymmetry. If the signal chain mediating spatial memory branched off after formation of phototropic asymmetry, it should also show a bell-shaped fluence-response relationship. The observed saturation curve (Fig. 5), however, points towards early separation (Fig. 10). Analysis of the interaction between photo- and gravitropically induced spatial memories (Nick et al. 1990) demonstrated that the branching between tropism and memory is situated before the confluence of photo- and gravitropic transduction (Fig. 10). For blue-light-induced memory this can be said more precisely: it must occur before formation of phototropic asymmetry.

*Cell versus organ polarity.* Phototropic asymmetry has been shown to be a systemic polarity, resulting from integration of locally perceived stimuli (Buder 1920). As pointed out above, memory formation branches off before this phototropic asymmetry evolves. The question arises of how the transverse polarity underlying the memory phenomenon is produced. Is it also a trans-organ polarity, stemming from comparing individual cell responses, or is it a cell polarity, as, for instance, appears

to be the case for gravitropic polarity (Nick et al. 1990)? In this context, Fig. 8a is worth a short discussion. It shows the pattern arising for phototropically superoptimal counterstimulation. Here, for long time intervals, the bending towards the first light pulse increasingly deviates towards the caryopsis as the fluence of the counterpulse rises (Fig. 8a, b). This reveals an interaction with the clinostat-elicited nastic curvature (Nick and Schäfer 1989). For phototropically optimal stimulation, nastic curvature is suppressed. If, however, the perceived amount of blue-light is phototropically superoptimal, this suppression looses its efficiency and the plants curve towards the caryopsis, producing the "aiming error" shown in Fig. 8b. More relevant to the question of polarity, however, is the behaviour for short time intervals, when plants curve towards the counterpulse (Fig. 8a). Here, there are two suprising observations: (i) the curvature is much larger than that the (phototropically superoptimal) counterpulse could induce, if given alone (Fig. 8a, dashed line); (ii) even a stimulus from above, if applied before the stabilisation point can evoke this strong negative bending (Fig. 8a, arrow). If the effect of the counterpulse were simple gradient addition, one should expect that different pulses, supposedly producing different gradients, should produce different outcomes. Apparently, the counterpulse does not necessarily have to be a gradient at all – even stimulation from above will do (Fig. 8a, arrow). The phototropically overoptimal counterstimulation, too, is expected to induce gradients which become smaller with increasing fluence. This becomes evident from the smaller curvature they induce if administered alone (Fig. 8a, dashed line). It is possible, in principal, to cause inversion of the initial gradient with strong light (which can even be symmetrical) by simple addition of local effects. The curvature resulting from such an addition does not increase linearly, but follows an optimum dose-response relationship ("overstimulation"). Such "overstimulation" has been repeatedly found in blue-light responses (Franck 1951; Hild 1977; Nick and Schäfer 1988a, 1989) and might well explain how even stimulation from above could reverse the bending towards the first pulse. However, Fig. 8a is much easier to understand if one assumes that the counterpulse actually reverses the effects elicited by the inducing pulse. Such an inversion has already been claimed, based on the fact that the curvature produced by the counterpulse given alone is consistently smaller than if it is preceded by the first pulse (Nick and Schäfer 1988b), although this first pulse is expected to outweigh the opposing pulse. Moreover, gravitropic curving has been reversed by a preceding blue-light pulse from above (Sailer et al. 1990). Inversion of a systemic polarity is difficult to imagine if one thinks about putative mechanisms. Inversion of cell polarity, instead, seems to be more likely. The example of gravitropic inversion by symmetrical blue light (Sailer et al. 1990) shows that, in fact, a response based upon cell polarity rather than systemic polarity can be reoriented by an action of light, which does not require a gradient. One could imagine, for instance, that individual cells perceive the counterpulse simply as a light-dark signal and respond by reorientation of their internal polarity. As soon as the counterpulse is saturating with respect to local light effects (Fig. 5), the resulting inverse gradient for short time intervals would be defined exclusively by the first stimulus, and become independent of the counterpulse gradient. This has been observed (Fig. 8a), thus favouring the assumption that the blue-light-elicited stable transverse polarity is cell based. Nevertheless, conclusive evidence cannot be extracted from these data, and it might well be that the question cannot be solved by mere phenomenology. However, defining the medium of transverse polarity (individual cells or whole organ) should become possible once cellular or subcellular markers for this polarity are available.

*Establishment versus stabilisation of transverse polarity.* The stimulus-summation experiments (Fig. 6a, b, Table 1) uncovered a discrepancy between the time, at which the memory escapes the counterpulse effect (apparent fixation point), and the actual fixation of spatial memory. Whereas apparent fixation was dependent upon the strength of the inducing stimulation (Fig. 5), actual fixation took place at about 2 h, for both phototropically optimal or only suboptimal inducing fluences (Table 1). This contradiction can be solved. One has to take into account that the apparent fixation points do not give the time at which the effect, triggered by the counterpulse, actually interacts with the transverse polarity (induced by the first pulse). One can only conclude that such effects of a counterpulse, administered at this time, cannot reach the interaction site before fixation occurs. When they eventually arrive at this point, polarity fixation is already under way. It is difficult to estimate the time elapsing between counterstimulation and actual interaction with the transverse polarity. The stimulus-summation schedule was supposed to shorten this time as much as possible and, in fact, could prolong the period in which polarity was reversible to about 2 h after induction. The dependence of apparent fixation times upon inducing fluence mirrors how the labile precursor of transverse polarity emerges. This polarity establishment is faster if the inducing pulse is stronger. Independent of polarity establishment, actual fixation occurs at about 2 h after induction. Its fluence-dependence is apparently different, since differences in the velocity of polarity establishment are not mirrored by differences in actual fixation (Fig. 5, Table 1). How the signal chain for polarity fixation relates to polarity establishment cannot be clarified with the present state of knowledge. It is only possible to make a distinction between establishment and fixation of transverse polarity. The temperature-sensitivity experiments point in the same direction (Fig. 9): in the first 20 min after induction, temperature-sensitive processes prevail (one might speculate about production or activation of essential factors); in the second, relatively temperature-insensitive phase, such factors might then be redistributed in a diffusion-like process. From 40 min after induction, this redistribution is accompanied by a second process, which is biochemical rather than merely physical. Again, the limits of the merely phenomenological approach are apparent. It is not possible to decide whether these changes in temperature-sensitivity belong to the establishment or to the fixation of transverse polarity.

*Predictions about the underlying mechanism.* The most straightforward model implies a gradient, the extent of which grows with time. The stronger the stimulation, the larger this gradient, the longer it takes for counteracting forces to reverse it. About 2 h after induction, this gradient is actually fixed (Table 1). Actual fixation is independent of the induction strength and probably involves efficient amplification mechanisms. In fact, this might be the step where the inferred all-or-none processes act.

Thus, the following conditions have to be met by a cellular marker of *transverse polarity:*

(i) It must involve an initially labile gradient. This gradient evolves with time and depends on the inducing fluence. A counterpulse can reverse this gradient for a certain time. The speed of this inversion is defined by the fluence ratio. In addition, the second pulse elicits the production of a factor essential for gradient establishment.

(ii) The outcome of this gradient interaction, irrespective of whether the inversion is complete or not, is fixed 2 h after induction. Fixation involves amplification mechanisms such as those suggested by Gierer (1981) for polarity induction in general. The outcome is merely qualitative (reversion, fixed symmetry). These amplification mechanisms evolve in parallel to gradient formation and interact with the latter 2 h after induction. Alternatively, they might follow gradient establishment and are triggered by a different signal chain, which needs 2 h for its completion.

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